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STUDIES ON THE REACTIONS OF PILOBOLUS TO LIGHT STIMULI

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(WITH TWELVE FIGURES)

The present investigation concerns itself for the most part with the problems of simultaneous stimulation. It was undertaken with a view to settling some of the problems suggested in a former paper on the light reactions of *Pilobolus* by Miss ALLEN and myself (1). Some of the objections inherent in the methods used in the earlier work were eliminated in the experiments here reported, and a study was also made of the reaction of single sporangiophores toward the light.

The work was begun under Dr. R. A. HARPER at the University of Wisconsin and completed under Dr. G. J. PEIRCE of Leland Stanford Junior University. I wish to acknowledge my indebtedness for their criticisms and suggestions. I wish also to express my gratitude to Dr. D. H. CAMPBELL for his interest in my work and for his courtesy in extending all the privileges of his laboratory. I thank Miss RUTH F. ALLEN, who began the study of the reactions of a single sporangiophore of *Pilobolus* toward the light and furnished the data taken on the evenings of May 18 and 19, 1910.

The present study of simultaneous stimulation by light is on the question of directive influence, and the literature discussed will concern that phase of light effect. The early work was carried on with light and gravity as the stimuli. NOLL (4) in 1892 pub-

lished his theory of "heterogene induction." He reviewed the earlier authors on the subject and described their conflicting results. According to NOLL'S theory, if an organism is subjected to two stimuli, one gives impetus to the other which carries out the reaction. One stimulus only is responded to. There is no resultant reaction toward the two stimuli. But when light and gravity work together, there is a change of geotonus due to light.

Recently GUTTENBERG (2) studied the simultaneous effect of light and gravity, using seedlings of *Avena sativa*, *Brassica Napus*, *Agrostemma Githago*, and *Helianthus*. He used what he called the compensation method. He tried different light intensities. With the higher intensities the reaction was toward the light alone. By gradually decreasing the intensities, GUTTENBERG found a certain light strength which just equaled that of gravity, and he obtained a resultant reaction between the two. A little weaker or a little stronger light gave a resultant reaction, varying according to the intensity of the light. GUTTENBERG considers this as evidence against NOLL'S theory of "heterogene induction."

RICHTER (5), working along the same lines as GUTTENBERG, came to quite different conclusions. For his experiments RICHTER used *Avena sativa*, *Vicia sativa*, *Vicia villosa*, *Brassica Napus*, and *Helianthus*. He followed GUTTENBERG'S method and in each case carried on a set of experiments in pure air and a similar one in impure air. He concludes that GUTTENBERG did not establish a resultant reaction between the effect of light and gravity by means of his compensation method, but that the latter's results were influenced by the impure air in which the experiments were performed. GUTTENBERG (3) followed this by a second paper in which he still maintained his former views. In this he repeated his own experiments, taking precautions to work under pure air conditions.

The experiments on simultaneous stimulation reported in this paper were performed with stimuli of the same kind, that is, they were light stimuli only. Before entering into a description of the work of simultaneous stimulation of *Pilobolus*, an account of some observations made on the reaction of a single sporangiophore of *Pilobolus* will be given.

Study of the reactions of the individual sporangiophore to a single light

In the earlier experiments (2), and also in those on simultaneous light stimulation in this paper, I was concerned with a large number of sporangiophores and with the net result of the reaction. This set of experiments was inaugurated for the purpose of following in detail the stages in the reaction of the individual sporangiophore toward light. The horizontal microscope was employed for this purpose.

A culture of *Pilobolus* in a 5-cm. flower pot was used. The pot was supported in an upright position. A thin glass Petri dish, measuring 5 cm. in diameter and 4 cm. in height, was placed over the top of the flower pot to keep the culture from drying. A 16-c.-p. carbon filament incandescent light was placed at a distance of 30 cm. from the culture, with the central point of the filaments 5 cm. above the level of the surface of the culture. The experiments were performed in the dark room and no other light had access to the culture. A horizontal microscope was placed with the tube on a level with the surface of the culture and at right angles to the direction of the light rays reaching the culture, so that any bending toward the light could be observed. A micrometer scale was placed in the ocular of the microscope in order to measure the change of position of the sporangiophore. In favorable cases several sporangiophores could be observed in the field of the microscope.

The first culture used in these experiments was put in place at 7:15 P.M. The sporangiophore had been exposed to the afternoon light and had grown straight out toward it, making an angle of 45° with the vertical. The culture was placed with the sporangiophores leaning away from the light, so that the angle between the light rays and the sporangiophore was about 135°. At the time when the experiment was set up, the young sporangiophores showed no signs of the sporangial swelling or vesicular bulb.

Two sporangiophores were observed during a period of 3 hrs. on the evening of May 18, 1910, and sketches were made at intervals during the reaction. The exact time when the reaction of the

first sporangiophore became perceptible was not determined. The reaction was distinctly visible at 7:45, 30 min. after it was exposed to the light. So far as could be seen, the curvature began at the tip of the sporangiophore, the tip bending as it grew. The tip distinctly started to curve upward at 7:45. This curve was somewhat more pronounced at 7:50. The tip was vertical, having moved through an angle of 45°. The radius of curvature was short. At 8:40 the tip had grown so that it was no longer vertical, but made a smaller angle with the incident light rays. At 9:30 the tip had grown around so that it pointed in the direction of the light. It had curved about 135° since the beginning of the observation. The curvature took place as the growth occurred; the curved end of the sporangiophore at this period was a well rounded hook. From

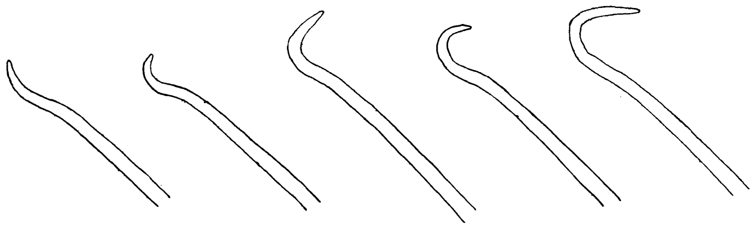

FIG. 1

this time on the tip grew straight toward the light. The last observation on this sporangiophore was made at 10 P.M. and showed a pronounced growth in the direction of the light.

Fig. 1 shows the stages that were sketched. The arrow indicates the direction of the light. The bending in this case had taken place always at the tip, the growing point of the sporangiophore. The older basal portion of the sporangiophore appeared to maintain the form and position which it had at the beginning of the experiment. If there was any change, it was so slight as not to be detected with the microscope.

The behavior of another sporangiophore under observation at the same time was as follows. The reaction was somewhat longer, no sign of curvature being noticeable until 8:00 P.M., 45 min. after the beginning of the experiment. The bending progressed slowly. At 8:40 the tip had curved through 45°, the curve being gradual.

At 9:15 the tip seemed to have stopped bending and to have grown straight. The angle made with the light was 60°. From 9:15 to 9:50 the increase in length was slight, and the older portion of the curve seemed to have become slightly more bent. At 10:15 the terminal sporangial swelling was well defined and the limits of the vesicular bulb could be discerned. The direction of the tip remained unaltered and formed an angle of 60° with the incident light rays (fig. 2).

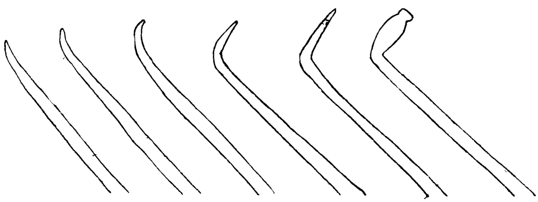

FIG. 2

A group of sporangiophores on the same culture as the two described above and subjected to the same stimulation from 7:15 P.M. until 9:55 were observed at 9:55. Three of them were still turned in the direction from which the afternoon light had come and away from the light used in the experiment. Apparently there was no response toward the light stimulus. In these three cases sporangium-formation had begun. The fourth sporangiophore had been subjected to the same conditions. The tip was curved through 135° and proceeded to grow in the direction of the light. The tip was still slender and pointed. The difference in length between this one and the other three was noteworthy. It exceeded the other three by the length of the portion beyond the bend where it turned toward the light. The sketches of the four sporangiophores as they were at 9:55 are shown in fig. 3.

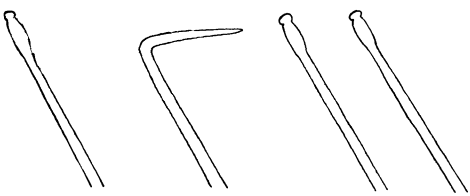

FIG. 3

At 8:15, May 19, 1910, an older culture was used. Sporangium-formation had started when the observations were begun. At the beginning of the experiment the five that were chosen for study were at different stages, the youngest showing the sporangium as a

small yellow knob, and the oldest having its sporangium full grown and turning black. They were all pointed in the direction of the afternoon light, making an angle of 135° with the incident electrical light rays. The five were watched closely from 8:15 to 9:55 P.M., the position of the tip of each being observed and recorded every 5 min. During this time their development continued normally, the sporangia of the younger ones swelled, turned gray, and then nearly black, and the vesicular bulbs of all increased in size. In no one of the five was there any sign of bending toward the light, although they were watched for 1 hr. and 40 min.

On the evening of May 21, 1910, observations were made on the reactions of three sporangiophores. The light was turned on at 7:38. The sporangiophores were inclined away from the direction of the light at an angle of 125°. The sporangial swelling in all of them was yellow. The vesicular bulbs had not begun to form. At 8:02 the sporangiophores had grown 1 mm. in length, but there was no change of position due to the presence of the light. The observation was continued until 9:20 P.M. The sporangiophores had not reacted toward the light, although they had continued their normal development.

At 9:20 P.M., January 19, 1911, observations were begun on six sporangiophores. They were in the same field. The light was turned on at 7:20 P.M. and observations were made for 2 hrs. One of the sporangiophores stood vertically. It was slender tipped and showed no signs of sporangial swelling. At 7:50, after 30 min. exposure to the light, the tips showed a very slight curvature. The reaction then stopped and the tip began to swell slightly. When the light was turned off, the sporangial swelling was distinct.

The remaining five sporangiophores at 7:20 stood at an angle of 135° to the incident light rays. All showed sporangial swelling, but the vesicular bulb had not started to form. There were no indications of response toward the light in any of them, although the sporangiophores continued their normal development throughout the experiment. At the close of the experiment the swelling of the vesicular bulb on all of them was just visible. Fig. 4 shows a sketch of these sporangia as they appeared at the beginning of the experiment.

At 7:27, January 20, 1911, the light was turned on a culture which had been exposed to the afternoon light, and observations were begun on five sporangiophores which were visible in one field. The first sporangiophore made an angle of about 80° with the direction of the light. It was slender tipped. At 7:58 the tip showed a slight increase in length and this portion was very slightly curved toward the light. At 8:23 there was a slight increase in curvature toward the light. At 8:42 the tip had curved through 35°, making an angle of 45° with the light. At 8:50 the curvature had increased so that the angle between the direction of light and the tip was only 25°. At 9:30 the tip was pointed directly toward the light. From this time the tip grew directly toward the light (fig. 5).

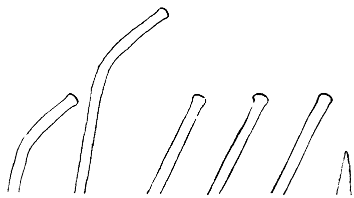

FIG. 4

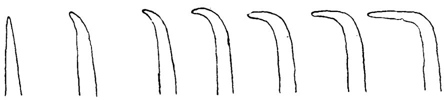

FIG. 5

The second sporangiophore observed was in much the same condition at the beginning of the experiment as the one just described, except that it was about 1 mm. longer. The reaction in this case was first noticeable at 8:00 P.M., the curve being barely visible. The curvature then proceeded more rapidly, and at 8:35 appeared more strongly than in the others in the field. At this time it had passed through an angle of 30°. At 8:42 the angle traversed was 45°. At 8:50 the angle made with the direction of the light rays was barely more than 10°, and at 9:13 the tip was directed straight toward the light and it continued in that direction until 9:50, when the observations were concluded. The portion beyond the curve was then about three-fourths of the length below (fig. 6).

FIG. 6

The third sporangiophore was inclined at an angle of 80° from the incident light rays and measured 4 mm. in length. It was slender tipped. Curvature was first visible at 7:58 and at 8:42 was still very slight. At 8:50 there had been a slight increase in length, although no further change in direction was noted. At 9:40 the curvature became more pronounced and at 9:50 the tip was pointing almost directly toward the light. At this time the curvature seemed to be arrested. No further observations were made on this sporangiophore (fig. 7).

FIG. 7

The fourth sporangiophore observed at the same time stood at an angle of 80° with the direction of the light. It was 2 mm. in length and the tip was slender and tapering. The reaction in this case was strikingly like that of the third sporangiophore just described. They were very near together (fig. 8).

The fifth sporangiophore measured 1 mm. in length at the beginning of the experiment. It was slender tipped and stood vertically from the surface of the culture. At 8:04 the tip had begun to curve. At 8:42 the tip had curved through an angle of 40°. At 9:13 it pointed in the direction of the light (fig. 9).

FIG. 8

Observations were begun on two sporangiophores on one culture at 7:40, January 21, 1911. They were slender tipped and made an angle of about 50° with the direction of the light. At 8:18 both showed new growth which was curved slightly toward the light. The curvature continued with the growth until at 8:50 the tip was directed straight toward the light. From that time until 9:50 when the observations were concluded, the sporangiophores grew in the direction of the light (fig. 10).

FIG. 9

A group of five sporangiophores was located in the field of the horizontal microscope at 8:40 P.M., November 2, 1911. The sporangiophores showed only slight differences in length and were inclined at an angle of 25° from the vertical. They were placed so that they leaned away from the light, making an angle of 95°. The sporangial swelling was just visible on the tips of all the spo-

rangiophores. They were observed continuously, but there was no sign of a bending toward the light until 3:00 A.M. Meanwhile, the sporangial swelling had increased in size and the vesicular bulb was barely visible. At this time the sporangiophores were curved slightly at some distance below the sporangium nearer the direction of the lamp. Observations were not made again until 4:30, when the sporangia were aimed directly toward the light and the vesicular bulb was distinct; the curvature was in the region immediately beneath the vesicular bulb. At 6:30, when the observations were concluded, the bulbs had swollen considerably. The sketches (fig. 11) show the development of one of the sporangiophores of the group. The development of all the others were remarkably parallel with the one described.

FIG. 10

Observations were begun using a second horizontal microscope on a sporangiophore at 9:00 P.M., November 2, 1911. The tip of the sporangiophore was just beginning to swell. The sporangium was standing vertical to the surface of the substratum. The light was placed at the angle above mentioned. The development of the sporangial swelling continued, but no reaction toward the light was visible at 11:35. Observations were made continually until 3:00 A.M., when the sporangium was rather well formed and the vesicular bulb barely visible.

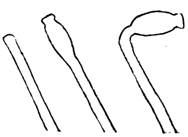

FIG. 11

On the evening of November 3, 1911, two sporangia were observed for the first time at 10:40. The first sporangiophore was yellow and rather blunt tipped, but as yet it did not show sporangial swelling. It stood at an angle of about 20° from the vertical, thus being inclined away from the light at an angle of 90°. There was no sign of a response toward the light at 12:00 P.M., but the tip then showed the sporangial swelling. At 2:05 A.M. the sporangiophore began to curve toward the light, the region of curvature being then located at some distance below the sporangial swelling. At 2:15 the curvature was more pronounced but still confined to the

same region. The bending continued until 5:30 A.M., when the tip of the young sporangium pointed directly toward the light. The entire portion between the region of bending and the young sporangium was swollen slightly, showing the beginning of the formation of the vesicular bulb. At 6:30 the vesicular bulb was large and turgid (fig. 12).

At the beginning of the observation the second sporangiophore in the same field of the microscope was somewhat longer than the first and the sporangial swelling was well formed. This sporangiophore also formed an angle of 90° with the light. The vesicular bulb was not yet visible. At 2:15 the sporangiophores had curved through an angle of 20° nearer to the direction of the incident light rays. The curvature in this case was also at some distance below the sporangium swelling, and the space between the two was

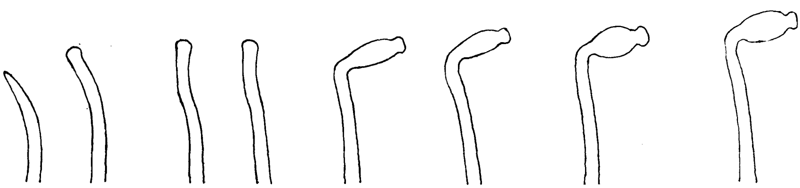

FIG. 12

beginning to show signs of the formation of the vesicular bulb. At 5:30 A.M. the tip of the sporangium was aimed toward the light. The vesicular bulb was still very inconspicuous. The bend in the sporangiophore was well rounded and had grown in length since the beginning of the curving. At 6:30 the vesicular bulb had swollen so that it exceeded the diameter of the sporangium by twice the diameter of the latter. At 8:00 the development appeared fairly complete. The sporangium was discharged between 9:50 and 10:00 A.M. (fig. 12).

From the foregoing experiments the following conclusions are evident:

1. Growth takes place at the tip in the young sporangiophore.
2. It is in the growing tip that heliotropic curvatures are formed.
3. In no case has a heliotropic curvature been observed during stages in early sporangium-formation.

4. Sporangium-formation may start during a reaction; in such cases the reaction is delayed for some time.

5. In case the reaction toward light is interrupted by sporangium-formation, it is resumed again a little before or about the time when the vesicular bulb is beginning to form.

6. After the sporangium is formed, the growing and curving portion is located immediately beneath the vesicular bulb.

The reaction of Pilobolus when exposed simultaneously to two equal sources of light

The effect of exposing a culture of *Pilobolus* simultaneously to two equal sources of white light was studied. For this purpose a light-proof box, measuring 120 cm. long by 60 cm. wide by 60 cm. high, was used. The box was made of pine and was painted a dull black on the inside. Running horizontally across one end, 20 cm. from the bottom, was an opening 10 cm. wide. Into the opening, which was prepared with rabbeted edges, was introduced a galvanized iron strip containing two openings 1 cm. in diameter and 9 cm. apart. The culture was placed at a distance of 25 cm. from the central point between the openings and on a level with them. It was placed with its surface vertical and facing the side of the box containing the openings.

The object of the experiment demanded that the light from the two openings be equal, but I know no methods of obtaining absolutely equal light intensities. We can only know that they are approximately equal. In order to obtain as nearly as possible equal illumination at the two openings, one light of measured intensity was placed equidistant from the two openings of the box and 40 cm. in front of it. Two mirrors were so adjusted that the culture intercepted the single spot of light formed by the convergence of the two sets of light rays. The light from either mirror was excluded from the opposite opening by means of the following device. At right angles to the edge of the box, along a line equidistant from the two openings, was placed an upright piece of board measuring 60 cm. high by 30 cm. wide by 1 cm. thick. At right angles to the first piece and parallel to the end of the box was nailed a second piece measuring 60 cm. high by 10 cm. wide by 5 mm. thick.

Both of the pieces were painted a dull black. The surface of the culture was covered with black paper, exposing a circular area 2 cm. in diameter. This small area was selected in order to exclude objectionable features such as unevenness in surface of culture, irregularity of distribution, etc. The number of sporangia was thus somewhat limited, but the undesirable features above mentioned were minimized.

This set of experiments was carried on in a dark room at the University of Wisconsin during April, May, and June 1910, under the direction of Dr. R. A. HARPER.

As previously described, a new set of sporangia matures daily and is discharged in the forenoon or early afternoon. The records of the results of the experiments were made daily in the late afternoon or evening. A glass plate fitting inside the box and against the openings caught the sporangia as they were discharged toward one or the other of the two lights.

The data were then recorded by means of a chart devised to meet the requirements of the experiment. The chart consisted of a large white sheet of paper divided by means of parallel lines into vertical strips 1 cm. wide. This is the principle of the Wolfhügel counter used by bacteriologists, and it was well adapted to the work in hand. The pieces of glass covering each of the 1-cm. openings fitted into the 1-cm. strips. In recording the data, the sporangia falling above and below the opening in the 1-cm. strip are recorded with those striking the opening. This is entirely fair, since, owing to the object of the experiments, we are concerned only with lateral distribution. Furthermore, our earlier experiments showed clearly the conditions of vertical distribution. The data for these experiments are recorded in table I.

In the first experiment 86 sporangia were discharged on the glass, 29 striking the vertical area containing the opening to the left and 25 that to the right. In the second experiment the total number was 60; 5 of these were on the area of the left opening and 20 on the right. In the third experiment 59 sporangia were counted on the glass, 5 and 18 being found on the left and right openings respectively. In the seventh all of the 22 sporangia discharged were fired toward the right opening, 10 of them striking the vertical

area containing the opening. Experiment 26 shows a total of 142 sporangia, 54 striking the vertical strip of the left opening, 30 that of the right opening. Experiment 27 shows 39 and 58 out of a total of 204 sporangia on the left and right openings respectively; and in experiment 28, 334 sporangia were discharged, 84 on the left and 78 on the right opening.

Throughout the series of experiments some of the sporangia failed to hit either opening. In experiment 1, 32 of the 86 sporangia discharged were of this sort. The distribution on either side of the two openings showed considerable variation. There were 9 sporangia in the 1-cm. strip to the left of the left opening and one sporangium in each of the next two strips. In the first 1-cm. strip to the right of the opening were 6 sporangia. On the left side of the right opening there were 11 sporangia in the first strip and 1 in the third; 3 sporangia were found in the first strip to the right of this opening.

In the second (table I), 3 sporangia struck the glass within 1 cm. to the left of the left opening. In the first, second, and third strips to the right of the opening were respectively 4, 2, and 1 sporangia. To the left of the right opening were 5 sporangia, all within the first strip. The number of sporangia in the first three consecutive strips to the right of the opening were respectively 18, 1, 1. The distribution and number of scattered sporangia in the remainder of the experiments showed about the same degree of variation as indicated by the complete data (table I).

In the foregoing experiments the number of sporangia which strike the openings does not appear especially large. These alone do not by any means give a complete conception of the accuracy of the total discharge of sporangia. A further knowledge of the distribution of these sporangia serves to correct the erroneous impression given by stating only the numbers that reach or do not reach the opening. The accuracy is very striking, for further consideration shows that 29 of the 32 sporangia that missed the opening in the first experiment were found within the 1-cm. strips on either side of the openings; only 3.5 per cent fell outside that area.

A perusal of the remaining data shows practically the same accuracy of discharge. The greater portion of the sporangia that

failed to strike the openings were found in the strips adjacent to the openings and only a very small percentage fell outside this area.

It is plain from these data that although there is much variation in the number of sporangia fired toward the two openings, the sporangia cluster about each of the openings and show no tendency to strike between the two. The sporangia are discharged, therefore, toward one or the other of the two lights. In other words, as shown by the earlier set of experiments, there is no resultant reaction due to the presence of the two lights. If there were, the sporangia would be for the most part between the two openings. It is clear, therefore, that this simple organism, when subjected simultaneously to two equal light stimuli, will respond to one of the two stimuli, to the complete exclusion of the other.

TABLE I

Total									Opening									Opening						No. of experiments
86	...	...	...	...	...	1	1	9	**29**	6	...	...	...	...	1	...	11	**25**	3	...	...	...	...	1
60	...	...	...	...	...	...	...	3	**5**	4	2	1	...	...	...	...	5	**20**	18	1	1	...	...	2
59	...	...	...	...	...	...	1	5	**5**	3	...	...	...	...	...	3	10	**18**	10	2	1	1	...	3
56	...	...	...	...	...	...	...	1	**2**	1	...	...	...	2	2	5	14	**14**	13	2	...	...	...	4
25	...	...	...	...	...	...	...	3	**8**	1	2	...	1	...	1	...	1	**7**	...	...	...	1	...	5
41	...	...	...	...	...	...	...	2	**9**	4	2	...	...	2	1	1	4	**10**	4	...	1	1	...	6
22	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	2	4	**10**	4	...	1	...	...	7
35	...	...	...	...	...	...	...	4	**7**	1	1	1	...	1	...	4	5	**7**	3	...	1	...	...	8
45	...	...	...	...	1	1	...	5	**13**	3	3	...	...	1	...	1	1	**11**	5	1	...	...	...	9
36	...	...	...	...	...	...	...	5	**7**	2	...	...	...	1	1	2	1	**12**	3	...	1	1	...	10
54	...	...	...	...	...	...	...	6	**3**	3	...	1	...	...	2	1	6	**21**	7	3	1	...	...	11
29	...	...	...	...	1	...	1	5	**3**	1	2	...	...	...	...	...	3	**13**	...	...	...	...	...	12
24	...	...	...	...	...	...	...	2	**4**	2	1	...	1	1	...	...	...	**11**	2	...	...	...	...	13
24	...	...	...	...	...	...	...	1	**9**	3	...	...	...	...	...	...	1	**8**	...	1	...	...	...	14
14	...	...	...	...	...	...	...	1	**4**	...	...	...	...	...	...	1	3	**4**	1	...	...	...	...	15
24	...	...	...	...	...	...	1	3	**7**	2	...	...	...	...	1	1	3	**6**	...	1	...	...	...	16
20	...	...	...	...	...	...	...	2	**3**	3	...	...	...	...	1	2	2	**3**	...	3	...	...	...	17
12	...	...	...	...	...	...	...	...	**4**	...	...	2	...	...	1	...	1	**2**	2	...	...	...	...	18
20	...	...	...	...	...	...	...	1	**6**	3	...	1	1	...	...	1	5	**1**	1	...	...	...	...	19
7	...	...	...	...	...	...	...	...	**2**	2	...	...	...	...	1	...	...	**2**	...	...	...	...	...	20
35	...	...	...	...	...	...	1	3	**10**	5	2	...	...	...	...	3	4	**7**	...	...	...	...	...	21
27	...	...	...	...	...	...	...	2	**8**	2	4	...	...	...	...	2	3	**5**	1	...	...	...	...	22
14	...	...	...	...	...	...	...	...	**5**	1	1	...	...	...	...	...	...	**4**	3	...	...	...	...	23
22	...	...	...	...	...	...	...	1	**4**	3	...	...	2	...	...	...	...	**7**	5	...	...	...	...	24
34	...	...	...	...	...	...	...	6	**12**	5	...	...	...	1	...	...	2	**6**	1	1	...	...	...	25
142	...	...	...	...	1	...	4	16	**54**	17	1	1	...	1	...	...	10	**30**	6	1	...	...	...	26
204	...	...	...	...	2	3	4	23	**39**	9	6	3	4	1	1	7	20	**58**	20	4	...	...	...	27
334	...	...	...	1	1	2	3	24	**84**	23	5	4	2	6	3	9	34	**78**	39	8	2	3	3	28
133	...	...	...	1	...	...	2	8	**43**	12	2	...	...	1	3	6	16	**32**	4	2	...	1	...	29
178	...	...	...	1	2	1	8	26	**52**	28	6	1	1	2	1	2	16	**21**	8	1	...	...	1	30
146	...	...	...	...	1	...	4	12	**25**	8	4	2	2	...	3	4	27	**44**	7	...	1	...	1	31
24	...	...	...	...	...	...	1	2	**2**	1	...	1	1	1	...	...	4	**9**	1	1	...	...	...	32
252	...	1	2	3	2	3	5	25	**25**	16	8	5	4	5	5	12	22	**44**	33	9	9	8	6	33
61	...	...	...	...	...	2	2	5	**2**	5	3	4	2	1	1	4	7	**13**	4	3	2	1	...	34
30	...	...	...	1	2	1	1	3	**2**	2	...	...	1	3	4	2	3	**2**	2	1	...	...	...	35
176	...	...	...	1	2	5	17	15	**9**	6	...	3	...	8	13	25	20	**19**	13	5	8	4	3	36

The sporangia, however, are not discharged in equal numbers toward the two openings. In the extreme case all might go to one or the other of the openings. It happened that in only one case (experiment 7, table I) did this occur, and then the total number was 22, the number being small and affording less chance for variation than would a larger number. The entire data thus show a great diversity of results as regards the number fired toward either opening. It is significant that the element of chance enters strongly into these results.

The fact that the sporangia do fire toward one or the other opening and in any degree of variation suggests further that although a large number of sporangiophores arise from one mycelium, they are not gregarious as regards physiological response toward light stimuli, that is, they act as separate individuals toward a light stimulus.

A further set of experiments was arranged to determine whether the sporangia to the right of the culture are discharged toward the right opening and those to the left of the culture to the left opening.

With the apparatus arranged as before, a second series of experiments was made with a thin plate of glass placed with its edge vertical to the middle line of the exposed surface of the culture. This glass would then intercept the sporangia discharged from the left side of the culture to the right opening and vice versa.

In the first experiment 2 sporangia struck each of the two openings, the number of sporangia in the consecutive 1-cm. areas to the left of the left opening are 8, 6, and 1; 3 sporangia were found on the 1-cm. area to the right of the opening. The first, second, and third 1-cm. areas to the left of the right opening contained respectively 1, 2, and 1 sporangia. The glass placed vertically at right angles to the surface of the culture received 7 sporangia on the left side and 15 on the right. The distribution of those on the left was as follows: 4 in the 1st cm. toward the culture, 2 in the 4th cm., and 1 in the 7th. The distribution of the sporangia on the right-hand side of the glass shows 7, 5, and 3 in the first 3 cm.

These data, together with those of the second and third experiments made in this series, are found in table II. These experiments tend to show that some of the sporangia on the right side of

the culture fire toward the left opening and vice versa. Some of this may be due to reflection from the glass.

TABLE II

The culture midway between and facing the two openings; a glass plate before the openings; a second glass plate at right angles to the surface of the culture. The data from the plates before the openings are given in the first part of the table; those from the plate at right angles to the surface of the culture are referred to by the marks *, †, and ‡.

Total								Opening									Opening							No. of experiment	Date
26	...	...	...	...	1	6	8	2	3	...	...	...	...	1	2	1	2	...	...	...	...	...	...	1*	6/15/'10 7:30 P.M.
7	...	...	...	...	...	...	1	...	...	...	...	...	2	...	...	1	2	1	...	...	...	...	...	2†	6/16/'10 7:35 P.M.
38	...	...	...	...	...	...	1	4	7	9	...	1	...	1	...	2	7	1	4	1	...	...	...	3‡	6/17/'10 7:20 P.M.
* Sporangia discharged on glass at right angles to surface of culture									4	...	...	2	...	...	1	...	...	Surface of glass to left of culture							
									7	5	3	...	...	...	...	...	...	" " " " right " "							
† Sporangia discharged on glass at right angles to surface of culture									6	3	...	...	...	...	...	...	...	" " " " left " "							
									2	1	2	1	1	...	...	...	1	" " " " right " "							
‡ Sporangia discharged on glass at right angles to surface of culture									1	1	...	2	1	...	...	...	1	" " " " left " " " " " " right " "							

The behavior of Pilobolus when given two equal sources of light but with the angle between the two sets of incident light rays varied

The box used for this set of experiments was made of redwood and measured 120 cm. long by 45 cm. wide and 35 cm. high. A hinged cover was so rabbeted as to be light-tight and was supplied with ice box catches in order to fasten it down tightly. At one end of the box, 14 cm. from the base, was an opening 9 cm. wide all the way across from side to side and so arranged with galvanized iron rabbets as to carry strips of galvanized iron 10 cm. wide which just closed the opening. These strips contained the openings through which the light was admitted to the culture. They were made of galvanized iron so that they would be thin enough to avoid shadows being cast by the rim of the openings. The openings were 1 cm. in diameter and their centers were the following distances apart: 1 cm., 2 cm., 3 cm., etc. The inner surface of the slide containing the opening was flush with that of the end of the

box, so that the glass plate on which the spores were caught fitted closely against the slide containing the openings. The openings not desired could be closed by means of two additional slides fitted in the groove from either end and outside the slide containing the openings. The inside of the box and of the strips was dull black.

The culture in this series was kept throughout the experiment at a distance of 23 cm. from the central point between the two openings. The surface of the culture was vertical and faced the slide containing the two openings. The exposed surface of the culture was 2 cm. in diameter. The light entering each of the openings was made to fall upon the exposed surface of the culture. The intensities of the light entering the two openings were equal or as nearly so as they could be made by measurement. The following device was followed in order to make the lights as nearly equal as possible. A single carbon filament incandescent lamp was placed at a distance midway between the two openings. By means of two mirrors placed at equal distances and equal angles one on either side of the lamp, the light was reflected through the opening to the exposed surface of the culture. In order to exclude the light of either mirror from the opposite opening a partition was set up in front of the box midway between the two openings, exactly similar to that used in the set of experiments last described.

With the change in the distance between the two openings it was necessary to change slightly the angles of the mirrors from the light in order that the spot of light reflected from the mirrors through the openings would strike the exposed surface of the culture. The condition of the experiments thus necessitated a slight change of light intensity from experiment to experiment, but the intensities of the light passing through the two openings at any one time were equal.

This set of experiments was performed in a dark room in the botanical laboratory of Leland Stanford Junior University.

In the first experiment, when the distance between the centers of the two openings was 5 cm., a total number of 38 sporangia were fired on the glass, 10 and 13 striking the left and right openings respectively. Only 2 of the 15 sporangia which failed to strike the opening were outside of the adjacent 1-cm. strips.

In the second experiment, with the center of the openings 4 cm. apart, 19 sporangia struck the glass; 10 were on the left opening, 7 on the right. Of the remaining 2 sporangia, 1 struck in each of the 1-cm. strips to the left and right of the left opening.

The third experiment shows a total discharge of 63 sporangia. Of that number 16 struck the left opening and 20 the right opening; 27 failed to reach either opening, but all of that number, excepting 3, were within 1 cm. of the opening.

When the distance between the openings was 14 cm., 50 sporangia were discharged, 15 striking each of the openings; 6 sporangia were counted within the first 1-cm. strip to the left of the left opening, and 1 in the third strip; 3 and 2 were within the first and second strip to the right of that opening; 5 sporangia struck within the 1-cm. strip to the left of the right opening, and 2 and 1 in the first and second strips on the right-hand side.

With a distance of 27 cm. between the two openings, 200 sporangia were discharged on the glass, 61 and 37 striking the respective openings to the left and right. The majority of scattered sporangia are again grouped around the two openings. The data for these experiments are tabulated in complete form in table III.

The results of the experiments in which the distances between the openings were varied agree with those obtained in the preceding set of experiments, where the openings were kept at the same distance throughout the set of experiments. The sporangia were fired with great accuracy toward one or the other of the two openings. The distribution of the sporangia about the openings varied in about the same degree as in the case just mentioned; and in the same way, the sporangia which are outside the vertical strips containing the openings are mostly within 1 cm. of one of the openings.

The reaction of Pilobolus when stimulated simultaneously by lights of different wave-length

The problems connected with the simultaneous stimulation of an organism by light rays of various wave-lengths offer an interesting field to the investigator. But I know of no way of accurately comparing lights of different colors as to the total amounts of

TABLE III

Distance between center of openings equals number of spaces between heavy figures plus one space.

Total																																			Number of experiment
38	...	...	...	...	...	...	1	...	...	1	...	...	2	**10**	3	4	**13**	4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
19	...	...	...	...	...	...	...	...	...	...	...	...	1	**10**	1	...	...	**7**	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2
63	...	...	...	...	...	...	...	3	...	...	...	3	**16**	7	1	1	4	**20**	8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3
20	...	...	...	...	...	...	...	...	...	...	...	4	**7**	1	...	...	...	1	**5**	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	4
76	...	...	...	...	...	...	...	...	...	...	1	8	**23**	9	1	1	...	...	7	**20**	6	...	...	...	...	...	...	...	...	...	...	...	...	...	5
137	...	...	...	...	...	...	...	...	...	2	9	**14**	6	...	...	...	...	1	17	**53**	32	2	1	...	...	...	...	...	...	...	...	...	...	...	6
143	...	...	...	...	...	...	...	1	1	2	6	**23**	7	2	1	1	...	1	1	26	**48**	17	2	1	3	...	...	...	...	...	...	...	...	...	7
104	...	...	...	...	...	...	...	...	1	14	**39**	13	3	...	...	...	...	...	...	11	**17**	5	1	...	...	...	...	...	...	...	...	...	...	...	8
50	...	...	...	...	...	...	1	...	6	**15**	3	2	...	...	...	...	...	...	...	...	...	...	5	**15**	2	1	...	...	...	...	...	...	...	...	9
114	...	...	...	...	...	2	...	9	**23**	12	2	1	1	...	...	...	...	...	...	...	...	3	13	**33**	11	4	...	...	...	...	...	...	...	...	10
91	...	...	...	...	...	1	2	**26**	14	2	...	...	...	...	...	...	...	1	...	...	...	...	4	10	**15**	12	3	1	...	...	...	...	...	...	11
161	...	...	...	...	...	...	11	**36**	16	5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4	24	**44**	16	5	...	...	...	...	...	12
133	...	...	2	...	6	21	**42**	18	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	1	7	**26**	6	...	1	...	...	...	13
66	...	...	...	2	...	**19**	3	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	8	**26**	5	...	...	...	14
200	...	1	7	22	**61**	20	4	2	2	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3	21	**37**	17	...	1	15
58	1	1	5	**14**	7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	7	**12**	10	...	...	16

radiant energy. The following experiments, therefore, are qualitative only; but I hope they will prove suggestive.

The different colors of the spectrum are represented in different proportions in the various incandescent lamps. These filaments are of standard make and the energy of the bulbs is measured in candle-powers. Thus by using bulbs of equal candle-power and current of known intensity we shall have somewhat comparable quantities.

Although this method is not all that could be desired, it has a very marked advantage over the colored solutions used by SACHS, and also the monochromatic glass plates that are so generally used in work on the effects of rays of various wave-lengths. The colored solutions absorb a large portion of the total energy emanating from the source, different solutions differing in this respect. In the case of the solutions (and it is also true of the colored plates) neither the intensity nor the energy of the lights can be compared. The incandescent lamps offer at least the advantage that they are of nominal commercial value; and with the advent of the knowledge of methods of comparison of intensities of colored lights, some exact idea of the intensities in the different parts of their spectrum may be obtained. Some study has been made to determine the distribution of the different wave-lengths in the different incandescent lamps, but so far it is insufficient for the present instance. It is known, however, that of the three incandescent lamps used in the experiments, the tungsten has the largest proportion of the actinic rays, the tantalum next, and the carbon least. The results with these filaments may thus serve to check up with those of the earlier work, in which the solutions and the plates of colored glass were used.

Experiments were made in order to test the relative efficiencies of different incandescent lamps in bringing about the reaction of *Pilobolus*. In these experiments the carbon filament, the tantalum, and the tungsten were compared. The experiments were performed in the dark room, using the redwood light-proof box already described. The openings were 1 cm. in diameter and the distance between them from center to center was 10 cm. The culture was placed 23 cm. from the point midway between the two openings.

The entire surface of the culture, which was 5 cm. in diameter, was exposed. Before each of the openings, at equal distances, was placed one of the two lights to be tested. The angle at which the lamps were placed was such that the spot of light from each lamp struck symmetrically the open surface of the culture.

The series of experiments was begun with a 32-candle-power carbon filament lamp before one opening and a 20-watt tungsten before the other. The first experiment gave a total of 92 sporangia; 31 sporangia were discharged toward the carbon filament and 61 toward the tungsten. In the second experiment 163 sporangia were discharged; 68 toward the carbon filament and 95 toward the tungsten. In the third experiment 387 out of a total of 784 sporangia went to the tungsten. Of the 1116 fired in the fourth experiment, 113 went to the carbon, 1003 to the tungsten. The next experiment shows a total discharge of 228 sporangia; 78 toward the carbon, 150 toward the tungsten. A new carbon lamp was put in the place of the old in the seventh experiment, but again a very much larger percentage was aimed toward the tungsten; 589 sporangia were fired, the ratio standing 168 toward the carbon as against 431 toward the tungsten. The data for this set of experiments are given in complete form in table IV. From this table a comparison of the accuracy of aim of *Pilobolus* toward the carbon and tungsten lamps can be made.

TABLE IV

Total								32-c.-p. carbon filament										20-watt tungsten						
92	...	...	...	...	...	...	6	**19**	6	...	...	...	...	...	...	3	7	**42**	9	...	...	...	...	...
163	...	...	...	...	...	1	3	**47**	14	2	1	...	...	1	1	3	15	**54**	20	...	...	1	...	...
784	...	1	1	...	...	6	64	**203**	86	15	6	4	2	2	5	3	40	**253**	88	3	...	1	1	...
1116	...	...	...	...	...	3	19	**69**	19	3	...	2	2	3	5	17	170	**607**	179	18	...	...	...	...
228	...	...	...	...	...	...	15	**47**	12	4	...	...	...	...	4	6	22	**101**	17	...	...	...	...	...
894	...	...	...	...	1	...	39	**111**	21	7	1	1	...	2	2	9	58	**503**	136	4	1	...	...	...
589	...	...	...	...	1	7	48	**78**	24	3	4	2	3	6	8	8	69	**258**	63	4	1	2	...	...

Of the 31 sporangia discharged toward the carbon lamp in the first experiment, 19 (61 per cent) struck the glass over the 1-cm. vertical strip containing the opening. Of the 61 sporangia

discharged toward the tungsten lamp, 49 (80 per cent) struck within the corresponding strip.

In the second experiment, of the 163 sporangia discharged, 68 were discharged toward the carbon filament, 47 (69 per cent) striking the 1-cm. strip over the opening; 95 were discharged toward the tungsten, 54 (56 per cent) on the strip over the opening. In this experiment, unlike the first, the larger percentage struck the strip over the opening in the case of the carbon filament.

In the third experiment, 387 sporangia were discharged toward the carbon, 203 (51.7 per cent) of them in the vertical strip containing the opening; 397 were discharged toward the tungsten, 253 (65.2 per cent) on the region of the opening.

The fourth experiment shows 69 sporangia, which is 61 per cent of the 113 sporangia discharged, toward the carbon, on the 1-cm. strip containing the opening; and 607 (61 per cent) of the 1003 sporangia fired toward the tungsten on the same region.

The remaining experiments of this series all show greater accuracy in the tungsten light than in the carbon filament light. With one exception, that of the second experiment, the discharge of the sporangia is more accurate toward the 20-watt tungsten used than toward the 32-candle-power carbon filament lamp, although the energy of the tungsten lamp is only half that of the carbon lamp.

Again, the percentages which strike the openings probably do not at first glance appear remarkable, but on noting in the first experiment that the 12 sporangia that did not strike the opening in the case of those fired toward the carbon light were all within 1 cm. of it, the accuracy is striking. Of the 19 sporangia which failed to strike the opening before the tungsten lamp, 16 were within 1 cm. of the opening and the remaining 3 were within 2 cm. of the opening.

In the second experiment, 21 of the sporangia fired toward the carbon light missed the vertical strip containing the opening; 17 of that number were within 1 cm. distance of the strip. Of the 41 sporangia that missed the opening in the case of those fired toward the tungsten, 35 struck within 1 cm. of the opening.

In the remaining experiments, most of the sporangia which failed to reach the opening in the strips fell within the first 1-cm.

strip on either side of them (table IV), showing the remarkable precision with which the sporangium is thrown toward a light.

A series of experiments was made to test the relative efficiencies of a tungsten and a tantalum lamp in bringing about a reaction of *Pilobolus;* 40-watt lamps of each kind were used. The tungsten was placed before the left opening, the tantalum before the right opening at a distance of 40 cm.

TABLE V

Total								Tungsten 40-watt										Tantalum 40-watt						
373	...	1	...	1	...	11	50	**137**	56	25	10	1	3	3	2	6	13	**31**	17	5	1	...	...	...
628	...	...	...	...	14	15	100	**243**	100	32	5	4	5	4	5	10	34	**34**	17	3	3	...	...	...
206	...	...	...	...	...	...	24	**71**	13	4	1	3	4	5	3	4	18	**39**	15	2	...	...	...	...
321	...	...	...	...	...	7	36	**104**	42	5	...	1	...	...	2	3	40	**61**	16	2	...	...	...	...
185	...	...	...	...	...	9	25	**61**	23	6	5	...	1	2	1	4	15	**24**	7	2	...	...	...	...
166	...	...	...	2	1	8	26	**67**	25	5	2	1	...	...	...	1	9	**13**	3	1	...	2	...	...
688	...	...	...	...	3	20	112	**291**	102	19	6	2	2	2	2	5	29	**47**	24	...	...	...	...	...
886	...	...	...	...	...	50	176	**363**	158	51	20	11	4	2	3	1	14	**22**	9	1	1	...	...	...
574	...	...	...	3	5	15	69	**248**	53	18	2	1	...	...	1	5	24	**43**	23	2	1	1	...	...
1091	...	...	...	6	20	46	156	**354**	164	46	21	8	7	8	13	20	40	**141**	51	15	5	...	...	...
464	...	1	1	5	2	21	81	**166**	71	29	17	3	2	3	3	8	7	**27**	9	4	2	2	...	...
367	...	...	...	...	1	17	82	**143**	28	25	15	7	1	...	1	4	8	**27**	7	1	...	...	...	...

In the first experiment, 373 sporangia were discharged on the glass, 294 toward the tungsten and 79 toward the tantalum. The second experiment showed a total number of 628 sporangia, with 516 and 112 toward the tungsten and tantalum respectively. The number of sporangia discharged in the third experiment was 206; of these, 118 were fired toward the tungsten and 88 toward the tantalum. The total number of sporangia in the fourth experiment was 321, 197 and 124 being fired toward the tungsten and tantalum. In the fifth experiment, 185 were discharged, 130 toward the tungsten and 55 toward the tantalum. The sixth experiment also showed a larger number had been fired toward the tungsten. The data for this set of experiments are found in table V.

The accuracy of aim toward the tungsten and the tantalum lamps was compared as in the preceding experiments. In the first experiment 46.6 per cent were discharged toward the tungsten, 39.2 per cent toward the tantalum. In the second experiment the

percentages fired toward the tungsten and tantalum were respectively 47 and 33.5. The third experiment shows 60.1 per cent before the tungsten and 44.3 before the tantalum; the fourth, 52.7 and 49.1 respectively; the fifth, 46.9 and 43.6; and the sixth, 48.9 and 44.8. The tenth and twelfth experiments show slightly larger percentages on the opening before the tantalum. The percentages figured out for the numbers striking either opening are given in table VI. The larger percentages strike the opening before the tungsten in these experiments. Most of the sporangia missing the opening strips in these experiments were found in the adjacent 1-cm. strips, as was the case in the foregoing experiments.

TABLE VI

Number of experiment	Percentages before tungsten lamp	Percentages before tantalum lamp	Number of experiment	Percentages before tungsten lamp	Percentages before tantalum lamp
1	46.6	39.2	7	44.3	42.7
2	47	33.5	8	41.3	40
3	60.1	44.3	9	59.5	43
4	52.7	49.1	10	42.9	47.6
5	46.9	43.6	11	41.7	40.9
6	48.9	44.8	12	44.2	56.7

In the next series of experiments a 40-watt tantalum was placed before one opening and a 20-watt tungsten before the other. The total number of sporangia fired in the first experiment was 1004; 731 were on the half of the glass toward the tantalum and 273 are on the half toward the tungsten.

The second experiment shows a total discharge of 407 sporangia, 329 on the tantalum as against 78 on the tungsten. The third and fourth experiments both show larger numbers on the tantalum. The data for this set of experiments are found in table VII. The accuracy of aim toward the two lights may also be obtained from the data in this table. Of the 731 sporangia discharged toward the tantalum lamp in the first experiment, 420 (54 per cent) struck the 1-cm. strip containing the opening. Of the 273 fired toward the tungsten, 149 (54 per cent) were on the corresponding strip. In the second experiment, the number of sporangia fired toward the tantalum lamp was 329; of these 161 (49 per cent) were in line with the opening. The total toward the tungsten was 78, with 37

(47 per cent) on the opening strip. In the third experiment, the ratio of the percentages of those on the 1-cm. strip containing the opening, in the case of the tantalum lamp, to that on the corresponding strip in the case of the tungsten is as 57 to 61. In the fourth experiment the accuracy of aim is very nearly the same toward the two lamps, although a greater proportion of the total number favor the tantalum lamp. The comparison of a 40-watt tantalum lamp against a 20-watt tungsten shows that a larger number of sporangia are discharged toward the tantalum, but that there is very little difference as regards the accuracy of aim toward the two lamps.

As in the set of experiments last described, a close examination of the data (table VII) reveals the fact that most of the sporangia that missed the vertical strips containing the openings were found within 1 cm. of them. An average of 7.8 per cent struck the glass more than 1 cm. laterally from the opening.

TABLE VII

Total								40-watt tantalum										20-watt tungsten						
1004	...	...	...	...	4	9	137	**420**	110	31	11	5	10	5	3	12	40	**149**	55	3	...	...	...	...
407	...	...	...	...	...	3	89	**161**	49	12	7	5	5	5	3	3	15	**37**	13	...	...	...	...	...
125	...	...	...	...	...	...	18	**40**	10	2	...	...	...	...	...	...	11	**34**	10	...	...	...	...	...
292	...	...	...	1	...	4	35	**111**	34	10	3	...	1	1	4	5	23	**45**	15	...	...	...	...	...

A 40-watt tantalum lamp was next compared with a 32-candle-power carbon filament with the results shown in table VIII. The first experiment gave a total of 96 sporangia, 36 of which were discharged toward the tantalum and 60 toward the carbon. The second experiment gave a total of 231 sporangia, with 164 fired toward the tantalum and 67 toward the carbon. In the third experiment 365 sporangia struck the glass, 268 over the tantalum and 96 over the carbon. In the fourth experiment, of the 157 sporangia fired, 98 were on the glass over the tantalum and 59 over the carbon. The fifth experiment showed a total discharge of 1039, 674 being found on the glass before the tantalum, 465 before the carbon. In the remainder of the experiments, as can be seen from

the data (table VIII), there is a larger percentage of sporangia over the tantalum. Thus, with the exception of the first experiment, the number discharged in the direction of the tantalum lamp surpassed that discharged toward the carbon.

TABLE VIII

Total								Tantalum 40-watt										Carbon 32 candle-power						
96	...	...	...	...	...	...	8	**23**	5	...	...	...	...	...	...	1	10	**40**	9	...	...	...	...	...
231	...	...	...	...	...	...	43	**79**	34	6	1	1	...	...	3	2	17	**32**	13	...	...	...	...	...
365	...	...	...	...	...	...	65	**136**	49	15	3	1	...	...	...	1	28	**48**	14	5	...	...	...	...
157	...	...	...	...	...	...	24	**57**	14	2	1	...	...	...	...	1	6	**40**	10	1	...	1	...	...
1039	...	...	...	...	...	7	116	**323**	107	15	3	2	2	2	1	15	91	**242**	105	8	...	...	...	...
76	...	...	...	...	1	2	12	**25**	1	1	3	...	...	...	...	2	6	**17**	6	...	...	...	...	...
404	...	...	...	...	...	1	35	**164**	29	1	...	...	...	2	1	1	26	**110**	31	3	...	...	...	...
1775	1	1	1	2	3	36	164	**748**	170	31	9	6	1	5	11	26	106	**283**	135	20	10	5	...	1
100	...	...	...	...	...	...	7	**47**	11	1	...	...	...	...	...	1	6	**23**	4	...	...	...	...	...
507	...	...	...	...	1	5	56	**154**	59	5	2	1	2	1	1	5	51	**121**	40	3	...	...	...	...
1442	...	...	...	1	3	8	208	**553**	246	24	8	2	2	7	6	28	85	**183**	63	6	2	3	...	2
813	5/11	4	4	10	15	20	58	**178**	77	15	15	17	16	18	25	31	65	**93**	71	18	18	9	5	11/4
174	...	...	...	...	1	3	29	**44**	8	2	1	1	1	3	2	7	15	**30**	22	7	...	...	...	...
249	...	...	...	1	...	3	20	**95**	18	1	1	...	...	...	1	3	30	**58**	17	1	...	...	...	...
111	...	...	...	...	...	...	11	**69**	17	1	...	...	...	...	...	...	2	**9**	2	...	...	...	...	...
104	...	...	...	...	...	...	10	**61**	17	1	...	...	...	...	...	...	3	**10**	2	...	...	...	...	...
184	...	...	...	...	...	...	19	**101**	19	1	...	...	...	...	...	...	7	**28**	9	...	...	...	...	...
194	...	...	...	...	...	...	22	**62**	27	1	...	...	...	...	...	...	6	**55**	20	1	...	...	...	...
123	...	...	...	...	...	...	12	**35**	8	...	...	...	...	...	...	2	10	**42**	14	...	...	1	1	...
1231	...	...	...	2	14	40	118	**266**	162	40	18	2	2	1	2	50	122	**257**	107	42	9	3	...	1
44	...	...	...	...	...	...	5	**19**	4	1	...	1	...	...	...	1	1	**11**	1	...	...	...	...	...
43	...	...	...	...	...	1	5	**16**	...	...	...	...	...	...	1	...	2	**12**	6	...	...	...	...	...
561	...	...	...	...	...	6	98	**146**	35	5	5	5	2	1	2	8	56	**122**	44	7	...	...	...	...

Of the 36 sporangia discharged in the direction of the tantalum lamp in the first experiment, 23 (63.8 per cent) struck the glass within the 1-cm. strip containing the opening. Of the 60 sporangia fired toward the carbon, 40 (66.6 per cent) struck within the 1-cm. strip containing the opening. In the second experiment, 79 (48.2 per cent) of the 164 sporangia that are discharged toward the tantalum are found in the 1-cm. strip over the opening; 32 (48 per cent) of the 67 discharged toward the carbon are on the corresponding strip. In the third experiment the percentages which strike the glass on the 1-cm. vertical strip over the opening before the tantalum and carbon filament lamps are respectively 50.7 and 50. In the fourth experiment 58 per cent of the sporangia fired toward the tantalum and 67.7 per cent of those fired toward the carbon filament lamp strike the strip containing the respective openings.

The above percentages striking the area of the 1-cm. opening before each of the two lamps compared are given, together with those computed for the remaining experiments, in table IX. The complete data from which the percentages were figured are found in table VIII.

TABLE IX

PERCENTAGES OF THE TOTAL NUMBER OF SPORANGIA DISCHARGED TOWARD THE TANTALUM AND CARBON FILAMENT LAMPS WHICH STRUCK THE 1-CM. VERTICAL STRIPS COVERING THE OPENING IN EACH CASE IN EXPERIMENTS 1–23 IN TABLE VIII

Number of experiment	Percentage before tantalum lamp	Percentage before carbon lamp	Number of experiment	Percentage before tantalum lamp	Percentage before carbon lamp
1..........	63.8	66.6	13.........	49.3	34.8
2..........	48.2	48	14.........	68.3	52.7
3..........	50.7	50	15.........	60.2	69.2
4..........	58	67.7	16.........	68.5	66.7
5..........	68.1	52	17.........	78.5	63.1
6..........	55.5	58	18.........	55.3	67
7..........	71.2	63.2	19.........	63.6	61.8
8..........	63.8	47	20.........	32.6	43.1
9..........	71.2	67.6	21.........	63.3	78.5
10..........	57	54.2	22.........	72.7	52.3
11..........	52.4	47.6	23.........	45.6	50.4
12..........	41.7	24.2			
			Average..	58.6	55.9

In this set of experiments the accuracy of discharge varies considerably. In some cases the discharge toward the tantalum is more accurate; in others that toward the carbon lamp is more so. On the whole, however, the discharge is a little more accurate toward the tantalum, but the difference is so small that it is practically negligible.

From the percentages in table IX, it stands out clearly that wherever there was a small percentage which struck the opening before one lamp, there was usually a comparatively small percentage on the corresponding area before the second light. In the first experiment, 63.8 and 66.6 were the percentages striking the two openings. In the second experiment, 48.2 and 48 per cent struck the openings. In some cases there is less uniformity. The eighth experiment shows 63.8 per cent on one opening and 47 per cent on the other. But on the whole, a small percentage on

one opening is usually accompanied by a corresponding percentage on the other. It might be suggested that this may be due to the general condition of the culture at the time of the experiment. The accuracy might be affected by food supply, moisture, temperature, and other factors of importance to the physiological condition of the sporangiophores at the time of stimulation.

Here, as in the previous experiments, the sporangia that strike outside of the opening are to be found for the most part in the first vertical strips to the left and right of the openings. An examination of the data (table VIII) will show a good proportion of the cases where all that were fired toward a single light are found on the opening or adjacent strips. Thus it is clear that the accuracy is much greater than it would appear from an examination of table IX alone.

Summary

Physiologists, in studying the reactions of plants to stimuli, have for the most part worked with phototactic organisms or organisms of considerable complexity, individuals in which there was a differentiation of tissues, where the cells in one portion of the body may receive a stimulus, another perceive it, and still another respond to it. Such a study has the disadvantage of dealing with too many factors and accompanying phenomena. In *Pilobolus* the reaction is marked and can be easily studied. A single cell receives the stimulus and responds to it. The protoplasm of the cell receives the stimulus, perceives it, and reacts. The accuracy of response of *Pilobolus* toward the light is remarkable, when we consider its size and the distance through which it throws its sporangia. The sporangiophore scarcely ever exceeds 1 cm. in length, and is usually somewhat shorter, while the distance through which it discharges the sporangium in most of the experiments is over 25 times that measurement. The accuracy of response and the nicety of organization of such a mechanism can well be appreciated from the study of such experiments. From such work the capacities of a single cell can best be realized.

The results of the experiments in which *Pilobolus* is stimulated simultaneously by two lights bear directly on NOLL'S (4) theory

of "heterogene induction." According to NOLL, the reaction of an organism to one of two stimuli excludes the effect of the second stimulus. His work was concerned with two very different kinds of stimuli, light and gravity. The reaction to the stimulus of light excluded any response to the stimulus of gravity. Recent workers, GUTTENBERG (2) and RICHTER (5), have interested themselves along the same lines. GUTTENBERG maintains that if the light stimulus be diminished sufficiently, a resultant reaction between light and gravity will occur; that NOLL and the earlier workers had used light that was too intense. To RICHTER'S (5) criticism that his results were due to impure air, GUTTENBERG (3) responded by further work under improved conditions and reached essentially the same results as before.

In experiments on simultaneous stimulation of *Pilobolus* by lights of the same kind or of different kinds, we are dealing, unlike either of the foregoing cases, with simultaneous stimuli of one kind, namely, light alone. It was possible to have the stimuli at least approximately equal, and it was possible to have the arrangement such that neither source of stimulation had any advantage over the other. Further, the organism worked with was a simple one, the reaction concerning only a single cell. And the net result of the reaction was shown so plainly in the distribution of the discharged sporangia that it seems impossible that any indefiniteness or uncertainty could be entertained as to the reaction. The sporangia clustered always about one or the other of the two sources of illumination. There was no sign of a resultant reaction. Even if the individual sporangiophore did not receive equal illumination from the two openings, if there were any resultant reaction, it would be expected that the sporangium would be found in a position between the two lights, depending on the ratio of their intensities, differences of composition, and the like. Thus, it would be expected that all of the sporangiophores would be located between the two sources of illumination. Such a condition did not obtain in any case where the two light stimuli were used, whether or not they were the same as far as distribution in the spectrum was concerned. The sporangiophore reacted to one or the other of the two stimuli. The results obtained at least suggest that one stimulus does not

affect the reaction to the other. To this degree the results are corroborative of NOLL's theory. But NOLL's theory, based on his work with light and gravity as stimuli, suggests a change of geotonus due to the presence of the light. In working with the two light stimuli, the reaction to one of the two stimuli to the exclusion of the other cannot be explained in this way, although here, as above, the plant is subjected to two directive influences.

Where the two simultaneous stimuli were of different kinds, gravity and light, NOLL believed that light may call forth certain changes in the plasma which, directly or indirectly, cause the reaction. He says that perception and reaction may rest on entirely different characters; but the reaction may be carried out by the same changes. In work where the two simultaneous stimuli are of the same character, as in the present experiments, the reaction is less complicated. Thus but two sets of changes of the plasma need be concerned with the response.

The above experiments show that there is no sign of a resultant reaction, but that they can in no way determine whether, so far as perception is concerned, there is any influence of one light on the other. According to NOLL's theory, a change of geotonus takes place, due to stimulation by light; that is, there is a change of sensibility to gravity as such due to the perception of light. The question arises whether, with the presence of two light stimuli of the same kind acting through the same time, there is a corresponding change of sensibility toward one light owing to the mere presence of a second of a similar kind. Apparently there must be some factor or factors somewhere in the organization of the plasma that brings about a total neglect of one stimulus and a complete reaction toward another of a similar nature, since the reaction is not a resultant one. It appears that NOLL's theory alone is insufficient to explain entirely the lack of resultant reaction to two directive forces when applied to an organism as contrasted with the results obtained by physicists in working with inanimate matter. The question, then, of what determines the reaction toward one light and a lack of response toward the second is still unsettled, and the explanation must be deferred to a time when more is known of the intricate mechanism and ultimate organization of the plasma of the cell.

In the experiment with the different incandescent filament lamps, as with the solutions and plates of colored glass used in the earlier work (1), *Pilobolus* fires its sporangia in larger numbers toward the lights in which the proportion of the blue rays is greatest. In other words, it is more responsive to actinic rays. The intensities in the different wave-lengths, as earlier mentioned, are not measurable; but the uniformity of response in favor of the source containing the greater proportion of actinic rays suggests the superiority of the more refrangible rays over the less refrangible rays in causing heliotropic curvatures. This question can be definitely settled only when the intensities of lights of different colors can be measured.

The energy given off by the source of light apparently does not compare in effect with the distribution of the same in different portions of the spectrum. In the experiments using a 20-watt or 16-candle-power tungsten lamp and a 32-candle-power carbon filament lamp the large majority of the sporangia went to the tungsten, although its total energy was but half that of the carbon. From this it is apparent that differences in distribution in the spectrum outweigh in effect the differences in the total energy of the two sources.

The set of experiments using a 16-candle-power tungsten against a tantalum of twice the number of candle-powers showed the discharge to be in favor of the tantalum. At first glance it appears that this contradicts the above results with the carbon and tungsten lamps, and suggests that the total energy of the source does play an important rôle in the results. However, on further consideration, it must be noted that the total number of actinic rays in a tantalum lamp of twice the intensity of the tungsten is probably greater than that in the tungsten. The solution of this point, of course, is bound up with the question of intensity and composition of the sources under discussion and cannot be taken with any degree of finality. With the tungsten lamp of approximately the same intensity as that of the tantalum, the discharge was in favor of the tungsten which emits a larger proportion of the blue rays. The difference as far as distribution in the different wave-lengths of the energy of the tantalum and tungsten lamps is not so great

as is the case of that in the carbon and tungsten lamps. This may account for some of the differences in the distribution of the sporangia in the two cases, when comparing the tungsten with the carbon and with the tantalum.

A comparison of the carbon and tantalum lamps shows again a majority of sporangia discharged on the opening before the tantalum lamp which contained the larger proportion of blue rays.

In the experiments with the rays of different wave-lengths, although the stimuli are both light stimuli, there is a marked difference in composition. We have then an extra factor to deal with; but, as in the experiments with the two nearly equal light sources, there is no sign of a resultant reaction. There is no sign of a change of aim toward one light owing to the presence of a second light. With the tendency of the sporangiophores to discharge toward the blue light, however, it is plain that there is no uniform aim of all the sporangia subjected to the two lights to go to the light having the larger proportion of the blue rays. Why does not *Pilobolus* always discharge toward the source of light having more of the actinic rays? The difference in the length of the light ray does bring a marked variation as regards the numbers fired toward the two sources. Still, some are fired toward the less favorable of the two sources.

The accuracy of aim toward the two lights might well be said to agree in general with that already found for the two light sources used in the above experiments. However, there is a noticeable difference in accuracy of aim toward the different filaments, and that for the most part is in favor of the light with the larger proportion of the more refrangible rays. With the solutions and glass plates used in the earlier work (1) there was a much greater difference noted. There is a probability that the smaller difference may be due to less difference in light intensity, to a smaller difference in composition, and also that there is a limit to the accuracy of response toward any source of stimulation, and that in aiming at the lights in use in these experiments they reached that limit, the less effective lamp being sufficient, or in some cases nearly so, to bring about as accurate a reaction as is possible to the plant.

The question as to what properties of the protoplasm cause it to be more sensitive to rays of one wave-length than to those of another remains unsolved, and, like that as to why it responds to one of two stimuli to the complete exclusion of the other, it must await a better knowledge of the organization of the plasma.

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